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**EFFECTS OF AQUEOUS EXTRACTS OF
COVER CROPS ON SEED GERMINATION:
INFLUENCE OF COVER CROP AGE AND
TOTAL PHENOLICS CONTENT**

Report No. 86-184-K

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Biologist
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ABSTRACT

Aqueous extracts prepared from shoot material of the cover crops white clover, yellow sweetclover, birdsfoot trefoil, meadow fescue, tall fescue, climax timothy, champ timothy and junco orchard-grass differentially inhibited the germination of black and white spruce, red pine and jackpine seeds. In general, extracts from older plants (i.e. eight to twelve weeks old) were more inhibitory than those from younger plants. The total phenolics content of extracts from birdsfoot trefoil, white clover, and tall fescue plants of various ages were significantly correlated with the inhibition of germination of white spruce or red pine seeds observed with the extracts. No such correlation was observed with extracts from yellow sweet clover or junco orchard-grass.

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34-548-031	834.1	October 2, 1986	86-184-K



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EXECUTIVE SUMMARY

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Cover crops are planted by Ontario Hydro on rights-of-way to establish a stable maintenance-free ground cover. They provide erosion control and resist the invasion of the right-of-way by undesirable woody species through competition and/or allelopathic means. Aqueous extracts, prepared from the shoots of the legumes, white clover (Trifolium repens L.), yellow sweetclover (Melilotus officinalis L.) and birdsfoot trefoil (Lotus corniculatus L.), and the grasses meadow fescue (Festuca elatior L.), tall fescue (F. arundinacea Schreb), junco orchard-grass (Dactylis glomerata L.), climax timothy (Phleum pratense L.), and champ timothy (P. pratense L.) exhibited allelopathic effects in that germination of seeds of one or more of the following species, black spruce (Picea mariana (Mill) B.S.P.), white spruce (P. glauca (Moench) Voss), red pine (Pinus resinosa Ait.) and jackpine (P. banksiana Lamb), was inhibited. In most cases, the older the plants used to prepare the aqueous extract, the greater the inhibition observed. There was a weak but significant correlation between the total phenolics content of extracts of birdsfoot trefoil, white clover and tall fescue and the inhibition of germination of white spruce or red pine seeds observed with the extracts. No such correlation was observed with extracts from yellow sweetclover or junco orchard-grass. Growing plants of tall fescue, junco orchard-grass and white clover under high light conditions in a growth chamber resulted in higher concentrations of total phenolics than in plants grown under low light conditions. However, there was no apparent

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correlation between the concentration of total phenolics and the inhibition of germination of the four seed species examined. The concentrations of allelopathic substances, total phenolics among them, in plants have been reported to be influenced by such factors as light quality and quantity, day length, mineral availability, temperature and water stress. Although the allelopathic substances in one species of cover crop may be aromatic in nature, such compounds might constitute but a fraction of all the aromatic compounds determined in the total phenolics assay. Thus any changes in the level of the minority compounds would be obscured by changes in the level of the majority.

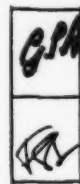


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To Dr. D. Mills
Director of Research

EFFECTS OF AQUEOUS EXTRACTS OF
COVER CROPS ON SEED GERMINATION:
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TOTAL PHENOLICS CONTENT

1.0 INTRODUCTION

After construction has been completed on Ontario Hydro's rights-of-way, mixtures of cover crops are commonly planted in an attempt to establish a stable maintenance-free ground cover that will provide both erosion control and resist the invasion by incompatible or undesirable species. This use of cover crops complements the selective vegetation control program adopted by Hydro at present, and ultimately contributes to reduced maintenance costs.

It is thought that the allelopathic properties of some or all of the components in the mixtures of cover crops provide some basis for the resistance to invasion of the right-of-way by incompatible woody species. Plants which exhibit allelopathic properties produce chemicals which inhibit or stimulate the germination, growth or development of other species of plants (Rice, 1984). Allelopathic effects are dependent upon the addition of a chemical to the environment and are thus separate from competition which involves the removal or reduction of some factor from the environment that is required by some other plant sharing the habitat (Rice, 1984). For a more detailed discussion of allelopathy see OHRD Report No 82-461-K.

Previous studies have shown that aqueous extracts, prepared from the shoots (stems and leaves) and roots of eighteen grasses and legumes, demonstrated allelopathic effects in that they inhibited the germination of black spruce (Picea mariana Mill), white spruce (P. glauca Moench), and jackpine (Pinus banksiana Lamb) seeds (OHRD Report No 84-19-K). Later studies revealed that most of the allelochemicals in junco orchard-grass (Dactylis glomerata L.), meadow fescue (Festuca elatior L.), climax timothy (Phleum pratense L.) and white sweetclover (Melilotus alba Desr.) were present in the shoots rather than the roots of the plants (OHRD Report No 84-456-K). Inconsistencies were

observed in the inhibitory nature of some of the plant extracts in these studies and it was suggested that the inhibitor content of such extracts was related to the maturity of the plants which were used to prepare the extract. In a previous study the potency of aqueous extracts prepared from herbage of the grass sorghum (Sorghum bicolor cv Bird-a-boo) in a seed germination bioassay was reported to be different relative to plant maturity, with herbage collected two and four weeks after planting being the most inhibitory (Lehle and Putnam, 1982). However, earlier work had revealed higher levels of inhibition by extracts of mature sorghum residues (Abdul-Wahab and Rice, 1967; Guenzi and McCalla, 1962; Guenzi et al, 1967). Rietveld (1975) found that extracts prepared from the foliage of Arizona fescue (F. arizonica) and mountain muhly (Muhlenbergia montana) collected in mid-September were not toxic to germinating ponderosa pine (P. ponderosa) seeds while foliage collected earlier in the year produced toxic extracts. While the influence of maturity of the plant material collected on the inhibition of seed germination in bioassays has been investigated in grass species, there have been no reports on such effects in legumes.

Most allelopathic agents are compounds termed secondary metabolites (Fraenkel, 1959; Whittaker and Feeny, 1971). Of the many thousands of secondary metabolites (see Swain, 1977) the aromatic compounds, such as phenols, phenolic acids, cinnamic acid derivatives, coumarins, flavonoids, quinones, and tannins, comprise the largest group. They are often referred to as 'phenolics' and have been identified as allelopathic agents in more instances than all the other classes of compounds combined (Rice, 1977). Although a colorimetric assay is available to quantify the total phenolics present in a plant extract, it is not known what changes there are (if any) in their levels during plant maturation. Many factors are known to influence the amounts of allelopathic substances in plants, phenolic compounds among them (Rice, 1984). For instance the synthesis of many classes of phenolics is known to increase with increased light intensity, and a variety of mechanisms has been proposed to account for this phenomenon (Smith et al., 1977).

The objective of the present study was to determine what effect plant maturity has on the level of inhibition observed in a germination bioassay performed with aqueous extracts prepared from the stems and foliage of several grass and legume cover crops. The levels of total phenolics present in a number of cover crops were also determined to discover whether there was a correlation between inhibition of the seed bioassay and the level of this group of secondary metabolites in the plant. The effects of different light levels during growth on both the inhibition of seed germination by aqueous extracts and the total phenolics content of several cover crops were also investigated.

2.0 MATERIALS AND METHODS

2.1 Plant Material

Seeds of eight cover crops (five grasses and three legumes) were obtained from Smith Seeds, Toronto. The five grasses were meadow fescue (Festuca elatior L.), tall fescue (F. arundinacea Schreb.), juno orchard-grass (Dactylis glomerata L.), climax timothy (Phleum pratense L.), and champ timothy (P. pratense L.). The three legumes were white clover (Trifolium repens L.), yellow sweetclover (Melilotus officinalis L.) and birdsfoot trefoil (Lotus corniculatus L.). The seeds were planted in 28x52 cm flats or 37 cm pots in a 1 and 1/2 to 1 mixture of shredded topsoil (native sandy loam - obtained locally) and peat moss (Plant Products, Bramalea). The seeds in pots were placed in the greenhouse and watered at regular intervals. The seeds in the flats were placed in a growth chamber maintained at 20/18° C day/night and a day length of 18 hours, and were watered every three days. Plants in the growth chamber were grown at high light ($500 \mu\text{Em}^{-2}\text{s}^{-1}$) or low light ($140 \mu\text{Em}^{-2}\text{s}^{-1}$) (see text). After four weeks in the growth chamber the above ground parts of the plants were harvested, dried in the oven at 60° C for one or two days, ground to a powder in a Wiley Mill and stored in plastic bags at -15° C. Plants grown in the greenhouse were harvested (above ground parts) from four to sixteen weeks after planting and dried, ground up and stored as described above.

Black spruce (Picea mariana (Mill.) BSP), white spruce (P. glauca (Moench) Voss.), red pine (Pinus resinosa Ait.) and jackpine (P. banksiana Lamb) seeds were obtained from the Ontario Ministry of Natural Resources (Angus Station) and stored at -2° C until required.

2.2 Preparation of Aqueous Extracts

Either 5 g or 10 g of ground plant material (5 g of the legumes and 10 g of the grasses) was combined with 100 mL of distilled water (pH 7.0 adjusted with KOH) and left for approximately 1 hour at 25° C. The residue was filtered off (vacuum filtration) and the extract made up to 100 mL with distilled water. The pH and conductivity (conductivity meter DP-05, Devon Products Corp, Ca.) of the extracts were measured.

2.3 Seed Germination

For each extract to be evaluated, three 9-cm petri dishes, each containing 40 black or white spruce, red pine or jackpine seeds on a filter paper were prepared. Seven mL of experimental extract or distilled water (control) were added to each petri

dish. The tops were placed on the petri dishes and they were placed in an incubator at 25° C in the dark. After seven or eight days the petri dishes were removed and the numbers of germinated seeds recorded. Data for seed germination were statistically compared by analysis of variance and multiple range tests (SNK) where indicated.

2.4 Extraction of Total Allelopathic Inhibitors Prior to Assay for Total Phenolics

A 1 g sample of each of the dried and ground birdsfoot trefoil samples, and the low and high light samples of tall fescue, white clover and junco orchard-grass was boiled and refluxed for 1 hour with 100 mL of 50% aqueous methanol (King and Heath, 1966). The residue was filtered off and the filtrate made up to 100 mL with 50% aqueous methanol. For all the remaining dried and ground samples, aqueous extracts were prepared as described above (2.2).

2.5 Assay of Total Phenolics

Either 0.5 mL or 1.0 mL of the 50% aqueous methanol extract or either 0.1 mL or 0.2 mL of the aqueous extract was placed in a 10 mL volumetric flask and 6.0 mL of distilled water added. After mixing 0.5 mL of the Folin-Denis reagent was added and the flask thoroughly shaken (Swain & Hillis, 1959). After 3 minutes, 1.0 mL of saturated sodium carbonate solution was added and the mixture made up to 10 mL with mixing. After 1 hour the optical density of the resulting blue solution was measured at 725 nm using a Pye-Unicam SP6 250 spectrophotometer against a reagent blank. On some occasions a precipitate formed and this was removed by centrifugation (5 minutes at 48,000xg) before readings were taken. When it was deemed necessary to centrifuge one or more samples, all the samples in the set were centrifuged. A calibration curve was constructed using tannic acid, and the total phenolic contents of the experimental samples expressed as tannic acid equivalents. The total phenolic contents of the extracted ground samples have been corrected, since only 0.5 mL or 1.0 mL of the 100 mL of aqueous methanol extract was assayed (similarly for the aqueous extract). The values given represent the mg tannic acid equivalents per g dry weight of sample.

3.0 RESULTS AND DISCUSSION

3.1 Inhibition of Seed Germination by Aqueous Extracts Prepared from Two Legume and Five Grass Cover Crops from Four to Twelve Weeks of Age

The effects of aqueous extracts prepared from seven cover crops, five grasses and two legumes, on the germination of black and white spruce, red pine and jackpine seeds are shown in Figure 1

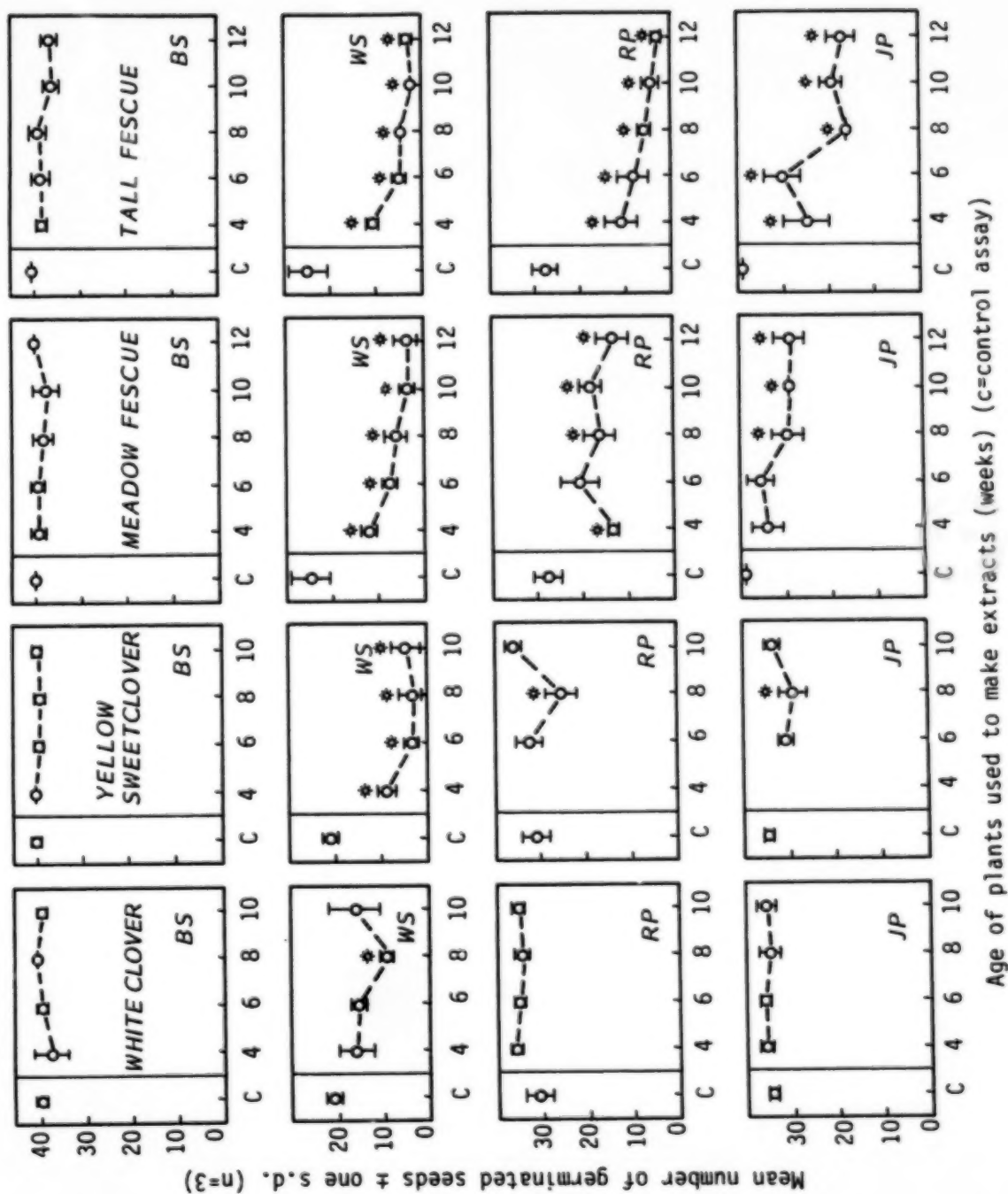


FIGURE 1

EFFECTS OF AQUEOUS EXTRACTS PREPARED FROM VARIOUS COVER CROPS ON THE GERMINATION OF BLACK SPRUCE (BS) WHITE SPRUCE (WS) RED PINE (RP) AND JACKPINE (JP) SEEDS POINTS MARKED WITH AN ASTERISK INDICATE THE DATA ARE SIGNIFICANTLY DIFFERENT FROM THE WATER CONTROL AS DETERMINED BY A ONE WAY ANALYSIS OF VARIANCE AND MULTIPLE RANGE TEST

and Figure 2. The harvested plants were between 4 and 10 or 12 weeks of age and had been grown in the greenhouse. Measurements of the pH and conductivity of each of the aqueous extracts revealed no obvious relationship between these parameters and the maturity of the plants in question (no data shown).

With all the extracts examined, germination of black spruce seeds was universally resistant, with only the 6 and 12 week extracts from junco orchard-grass causing slight but statistically significant inhibition (Figures 1 and 2). However germination of white spruce seeds was invariably inhibited by almost all the extracts examined, and the level of inhibition appeared to increase as a function of the age of the plant material extracted. The germination of both red pine and jackpine seeds was not inhibited by some extracts e.g. white clover extracts (Figure 1). In other cases, e.g. tall fescue extracts with red pine (Figure 1), all the extracts of a particular cover crop species caused inhibition and the degree of inhibition increased with the age of the plant material used to make the extracts. Although no one series of extracts from one cover crop species was found to consistently inhibit the germination of all four seed species, some groups of extracts were found to inhibit the germination of three of the species. Extracts from tall fescue were consistently inhibitory in the white spruce, red pine and jackpine bioassays, and in each case the extracts from the older plants were the more inhibitory.

In general, the extracts from the five grass species examined were found to be more inhibitory than the extracts from the two legume species (compare white clover and yellow sweetclover results with those of the remaining species - Figures 1 and 2). Differences in the inhibitory nature of extracts made from grass species have been described previously, eg the species Arizona fescue and mountain muhly (Rietveld, 1975) and sorghum (Guenzi and McCalla, 1962; Guenzi et al, 1967; Abdul-Wahab and Rice, 1967; Lehle and Putnam, 1982) and although there does appear to be some connection between age and the inhibitory nature of the aqueous extracts prepared, it is sometimes difficult to compare the results from different workers because the precise age of the plants is not described. It should be remembered that not all the plant is the same age, since during the growing season new tissue is formed all the time, and thus any plant will be a mixture of new and old(er) tissue. For completeness, plant samples might be collected at regular times up to the point the plant has stopped growing and has begun to die back (senescent), and bioassays could be performed on the aqueous extracts prepared. Alternatively, aqueous extracts could be prepared from new and old leaves from the same plant.

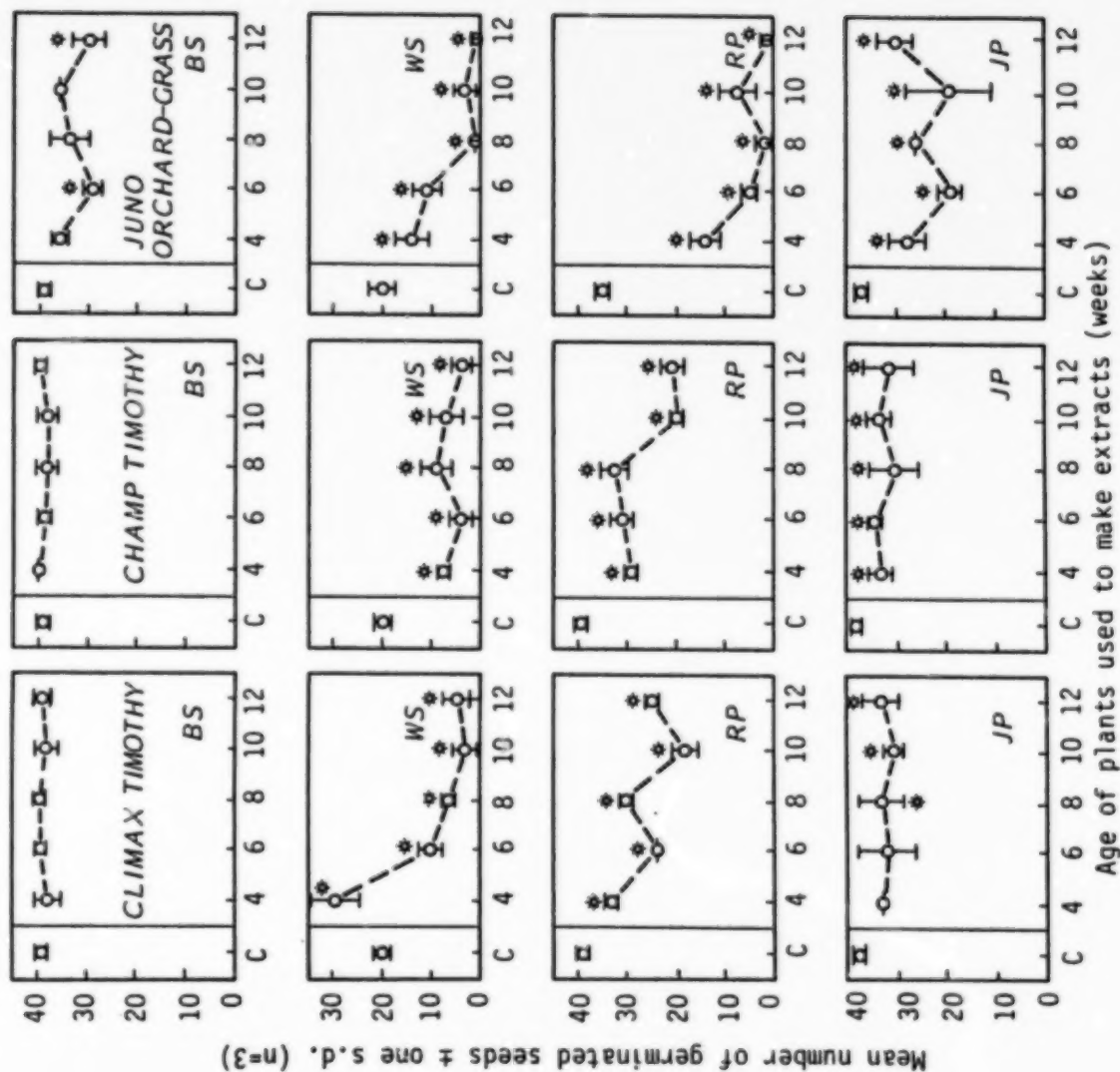


FIGURE 2

EFFECTS OF AQUEOUS EXTRACTS PREPARED FROM VARIOUS COVER CROPS ON THE GERMINATION OF BLACK SPRUCE, WHITE SPRUCE, RED PINE AND JACK PINE SEEDS ABBREVIATIONS AND OTHER DETAILS AS DESCRIBED IN FIGURE 1

Of the two legume species examined yellow sweetclover did show an increase in the level of inhibition of seed germination as a function of plant age, but only with white spruce as the bioassay species (Figure 1). There have been no previous reports on the influence of age of a leguminous plant on the inhibitory properties of extracts from those plants in a seed germination bioassay.

3.2 Correlation Between the Inhibition of White Spruce Seed Germination by Aqueous Extracts Prepared From Birdsfoot Trefoil Plants From Four to Sixteen Weeks of Age, and Total Phenolics Content of the Plant Material

The effects of aqueous extracts prepared from birdsfoot trefoil plants, collected up to 16 weeks after planting, on the germination of white spruce seeds are shown in Figure 3. Extracts prepared from the older plants appear to be more inhibitory, although the only extract to cause a significant inhibition was from 16 week old plants. No such inhibition was observed with the other three seed species used: black spruce, red pine and jackpine (data not shown).

Birdsfoot trefoil is a legume, like white clover and yellow sweetclover, and it has been reported that legumes contain the secondary metabolite coumarin. It has been proposed that such chemicals are responsible for the inhibition of seed germination observed with legume extracts (Lawrence and Kilcher, 1962; Whittaker and Feeny, 1971). The total phenolics content of the dried birdsfoot trefoil material collected after 4 to 16 weeks growth is also shown in Figure 3. Over the course of the growth period the level of total phenolics extracted with methanol from the dried material increased by almost threefold. An attempt to relate the total phenolics content of birdsfoot trefoil material with the inhibition of germination of white spruce seeds by an aqueous extract of same revealed a direct correlation between the two. The correlation coefficient, r , of 0.63 (see Table 1) was significant at the 1% level. Since the same aqueous extract did not inhibit the germination of black spruce, red pine or jackpine seeds, no such relationship was apparent with these species.

While methanol has been used extensively in the extraction of allelopathic agents from dried plant material, perhaps the oldest and most commonly used solvent has been cold water (see Putnam, 1978). This latter method is thought to simulate the natural release of compounds by the action of rain on standing or fallen plant material (Putnam, 1978). The tree seed bioassay experiments described previously used extracts made with cold water and it might be more appropriate to perform the total phenolics assay on samples from these extracts rather than from

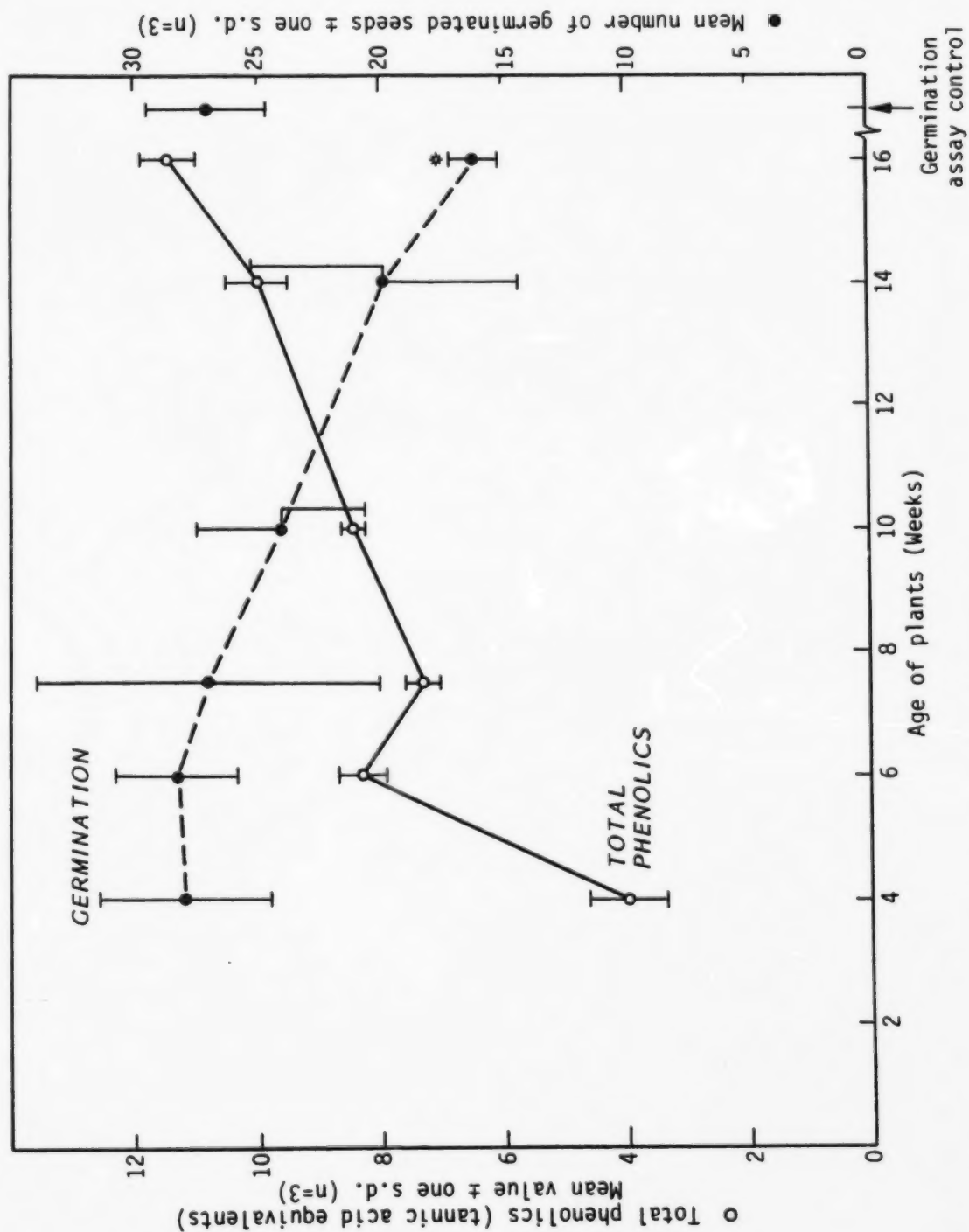


FIGURE 3

EFFECT OF AGE OF BIRDSFOOT TREFOIL PLANTS ON TOTAL PHENOLIC CONTENT
AND INHIBITION OF WHITE SPRUCE GERMINATION
GERMINATION VALUES MARKED WITH AN ASTERISK ARE SIGNIFICANTLY DIFFERENT FROM THE CONTROL

Table 1

Correlation Between Total Phenolics Content of Extracts
From Cover Crops and The Inhibition of Germination
White Spruce and Red Pine Seeds by Those Extracts

Cover crop Species†	Number of Observations	Bioassay Species	
		White spruce - r (correlation coefficient) -	Red Pine
Birdsfoot trefoil††	18	0.63**	n.d.
White clover	18	0.61**	0.57*
Yellow sweet-clover	12	-0.06	-0.44
Juno orchard-grass	18	-0.15	0.28
Tall fescue	18	0.67**	0.73**

† Extracts were prepared from birdsfoot trefoil plants, 4.0, 6.0, 8.0, 10.0, 14.0 and 16.0 weeks old; from yellow sweetclover plants 8.7, 10.1, 13.8 and 15.2 weeks old; from the remaining three species 6.0, 8.7, 10.1, 11.2, 13.8 and 15.2 weeks old.

†† Phenolics extracted with methanol, with all other species the aqueous extract was assayed.

* Significant at the 5% level

** Significant at the 1% level.

n.d. Not Determined

methanol extracts. The two extraction methods were compared in a series of experiments in which the same weight of dried plant material of junco orchard-grass, tall fescue, birdsfoot trefoil and yellow sweetclover was extracted with methanol or water and the total phenolics content of the resulting extracts determined. The water extraction method released 17, 30, 22 or 27% of that released by the methanol for the four species respectively.

3.3 Correlation Between the Total Phenolics Content of Extracts from Various Cover Crops and the Inhibition of Germination of White Spruce and Red Pine Seeds by Those Extracts

The correlations between the total phenolics content of aqueous extracts from white clover, yellow sweetclover, junco orchard-grass and tall fescue, and the inhibition of germination of white spruce and red pine seeds caused by those extracts are given in Table 1. These aqueous extracts were prepared from different sets of plant material to those described in Figures 1 and 2. The results from these later seed bioassays and total phenolics assays are not shown. In general, the increases in total phenolics in these extracts were less than the increase observed with the birdsfoot trefoil extracts used earlier (see Figure 3). In these later experiments, between 4 to 6 extracts, prepared from plants of different ages, were used for each species. For both white clover and tall fescue, the correlation coefficients obtained with both white spruce and red pine seeds were significant at the 5% level or the 1% level. With both yellow sweetclover and junco orchard-grass, the correlation coefficients obtained with both white spruce and red pine were not significant and in some instances were negative.

The largest correlation coefficient obtained was 0.73 for tall fescue extracts using red pine as the bioassay species. Such a correlation coefficient yields a coefficient of determination, r^2 , of only 0.53. Thus only 53% of the variance of inhibition in the bioassay is accounted for by variation in the total phenolics content of the extract used in the bioassay. For the remaining correlation coefficients given in the table, the coefficients of determination would be even smaller. These analyses suggest that the total phenolics assay may only provide limited information as to the phytotoxicity of the aqueous extracts prepared from dried cover crop material.

The total phenolics assay used in this study is a non-specific assay, in that it does not distinguish between the aromatic compounds, such as phenols, phenolic acids, cinnamic acid derivatives, coumarins, flavonoids, quinones and tannins, present in the methanol and aqueous extracts. The assay merely measures the cumulative amount of all these compounds. Some of these

compounds may be more inhibitory than others. If such compounds are only present in small amounts, then changes in the amounts of these compounds as a function of age of the plant material may be masked by changes in the amounts of compounds which are not phytotoxic.

3.4 Inhibition of Seed Germination by Aqueous Extracts Prepared From Tall Fescue, White Clover and Juno Orchard-Grass Plants Grown at High and Low Light Intensities, and the Total Phenolics Content of the Plant Material

There have been reports that light quality and intensity influence the quantity of inhibitors present in various plant species (see Rice, 1984). In a series of experiments conducted at an early stage of this study, tall fescue, white clover and juno orchard-grass plants were grown in growth chambers at high or low light levels and the total phenolics content of the dried plant material (extracted with methanol) and the inhibition of germination of the four seed species by aqueous extracts determined.

For each of the three cover crop species studied, the concentration of total phenolics in plants grown in high light was approximately twice that of plants grown at low light (Table 2). The legume, white clover, had the highest total phenolics concentration of all species grown at high light, while the two grass species, tall fescue and juno orchard-grass had remarkably similar concentrations for plants grown at high light and for plants grown at low light. Aqueous extracts prepared from the various species did not show consistent trends when the germination of the four seed species was examined.

Germination of white spruce seeds was inhibited by all the extracts examined while germination of jackpine seeds was unaffected. Black spruce seed germination was significantly inhibited by two low light extracts, but the magnitude of the inhibitions observed was very small. This experiment did show that the levels of total phenolics could be affected by growth conditions, but a clear relationship between these levels and inhibition of germination was not demonstrated. Recent studies have shown that the allelopathic substances in tall fescue are primarily non-aromatic in nature, being acids such as lactic, succinic, malic, citric, shikimic, glyceric, fumaric and quinic (Peters and Luu, 1985) and thus phenolic compounds may be important allelopathic substances in some species and not others.

Many factors are known to influence the amounts of allelopathic substances in plants (Rice, 1984). Ionizing radiation, ultraviolet radiation and red and far-red light have all been shown to influence the concentrations of phenolic compounds in crop

TABLE 2

Effects on Seed Germination by Aqueous Extracts Prepared from Three
Cover Crops Grown at Different Light Levels, and the Total
Phenolics Content of the Dried Cover Crop Material Extracted With Methanol

Extract	Light	Total Phenolics Content†	Bioassay Seed Species+			
			Black Spruce	White Spruce	Red Pine	Jackpine
Control	-	-	40.0±0.0	35.0±1.0	35.0±1.0	31.0±1.0
Tall Fescue	Low	8.8±0.3	37.3±0.6*	25.0±2.6*	35.0±1.0	20.3±12.5
Tall Fescue	High	16.7±0.1	39.7±0.6	16.0±4.2*	33.7±2.1	24.3±1.2
White Clover	Low	11.9±0.3	38.3±0.6*	29.0±1.7*	35.3±0.6	27.7±6.8
White Clover	High	28.7±0.3	39.7±0.6	28.0±3.6*	36.3±1.5	31.0±1.0
Juno Orchard-Grass	Low	8.8±0.3	38.7±0.6	22.0±3.0*	9.0±6.2*	16.7±7.6
Juno Orchard-Grass	High	16.7±0.1	39.0±1.0	24.7±1.5*	24.3±11.8	16.7±4.9

+ Results given are the mean number of germinated seeds ± one s.d. (n=3).

† Results given in tannic acid equivalents, are the mean ± one s.d. (n=3) after assay of the methanolic extract prepared from the dried cover crop sample.

* Values within a column so marked are significantly different, at the 5% level using a multiple range test, from the control value at the top of that column.

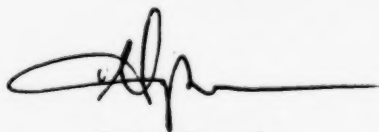
plants. In both the greenhouse and growth chamber, the amount of ultraviolet radiation is minimal, and supplementing greenhouse light with ultraviolet light has been shown to increase the chlorogenic acid content of tobacco from 0.41 to 2.52%, the latter value then approached the concentration found in control plants grown out-of-doors, of 2.72% (Frey-Wyssling and Babler, 1957). Day length, temperature and water stress, and deficiencies of the minerals boron, calcium, magnesium, nitrogen, phosphorus, potassium, and sulphur, have all been shown to influence the concentration of allelopathic compounds (Rice, 1984). It is apparent that for any particular species of plant growing at a specified location the influences on the concentration(s) of allelopathic compounds present in that plant are complex.

CONCLUSIONS

1. Aqueous extracts prepared from the dried shoots of the cover crop species yellow sweetclover, birdsfoot trefoil, meadow fescue, tall fescue, climax timothy, champ timothy and junco orchard-grass were shown to inhibit the germination of one or more of the four seed species examined. In general extracts prepared from older plants (8, 10 and 12 weeks old) were more inhibitory than extracts prepared from younger plants.
2. There was a statistically significant correlation at the 5% level between the concentration of total phenolics in extracts prepared from birdsfoot trefoil, white clover and tall fescue plant material and the inhibition of seed germination of tree species such as white spruce or red pine by aqueous extracts prepared from that material. No such direct correlation was observed with extracts from yellow sweetclover or junco orchard-grass.
3. Although the concentration of total phenolics in tall fescue, white clover and junco orchard-grass grown in the growth chamber was higher in plants grown in high light conditions as opposed to low light conditions, there was no consistent relationship between total phenolics content and the inhibition of germination in the seed bioassays.
4. Although the concentration of total phenolics in a plant may be an indication of the allelopathic potential of such a species, there may be no such relationship in other

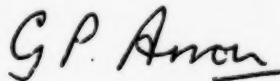
species, where either non-aromatic compounds are the primary allelopathic substances or those aromatic compounds that are allelopathic constitute but a small fraction of the total determined in the total phenolics assay.

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